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EXPERIMENTAL STUDIES ON THE ENDOCRINOLOGY AND REPRODUCTIVE BIOLOGY OF THE VIVIPAROUS POLYCHAETE ANNELID, *NEREIS LIMNICOLA* JOHNSON [1]

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The reproductive biology of relatively few Northeastern Pacific nereids has been described: *Nereis limnicola* (as *Neanthes lighti*) (Smith, 1950), *N. vexillosa* (Johnson, 1943), *N. grubei* (Reish, 1954; Schroeder, 1968), *N. caudata* (Reish, 1957), and *Micronereis nanaimoensis* (Berkeley and Berkeley, 1953). Gould and Schroeder (1969), Schroeder (1967, 1968), and Baskin (1970) have reported on experimental studies related to endocrine control of reproduction and development.

The reproductive endocrinology of *N. limnicola* is of interest for a number of reasons. This viviparous species has a close relationship with, and is virtually indistinguishable from, the oviparous *Nereis diversicolor* (Smith, 1958), the subject of previous studies on the hormonal control of reproduction (Durchon, 1952; Clark and Ruston, 1963; Durchon and Boilly, 1964; Durchon and Dhainaut, 1964; Durchon and Dhainaut-Courtois, 1964; Durchon and Porchet, 1970). The embryology and reproductive biology of *N. limnicola* has been studied by Smith (1950). These viviparous worms are self-fertilizing hermaphrodites; fertilization occurs in the coelom, where development proceeds until the larvae are 4–5 mm in length and have approximately 20–30 pairs of parapodia. Parturition occurs by ruptures of the body wall of the adult. This reproductive pattern is unique among nereids (Smith, 1958) and raises the possibility of endocrine regulation of viviparity.

Both somatic and gametic maturation are known to be hormonally controlled in nereids (see reviews by Clark, 1965, 1969; Hauenschild, 1965; Durchon, 1967). The primary oöcytes are shed from a proliferative epithelium and grow within the coelomic cavity (Dales, 1950; Durchon, 1952). Once the oöcytes have reached a critical diameter, removal of the brain (supraesophageal ganglion) may result in a phase of rapid oöcyte growth, although this response is variable in different species (Durchon, 1952, 1956; Clark and Ruston, 1963; Hauenschild, 1965, 1966; Dhainaut and Porchet, 1967; Malecha, 1967; Schroeder, 1968). Typically, somatic and gametic maturation coincide, whether somatic maturation consists of metamorphosis into the epitokous heteronereid form, or in the less spectacular modifications characteristic of atokous species, and it is thought that both processes are con-

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trolled by the declining titer of a single hormone of presumed cerebral origin (Golding, 1967c). However, several observations by Smith (1950), as well as by the present authors indicate that the temporal relationships of the gametic and somatic aspects of maturation in *N. limnicola* differ from the typical nereid pattern.

The source of the maturation-inhibiting hormone in nereids has been presumed to be neurosecretory cells of the supraesophageal ganglion (see Gabe, 1966; Golding, 1967b; Dhainaut-Courtois, 1968b, for reviews). More recently, however, it has been suggested that the infracerebral gland, a possible neuroendocrine complex on the ventral surface of the cerebral ganglion, might be the source of this factor (Dhainaut-Courtois, 1968a; Golding, Baskin and Bern, 1968). This complex is composed of an epithelium that contains two principal cell types: (1) the prominent *a* cells, which in transverse section give the gland the configuration of a columnar epithelium and whose cytoplasm is devoid of electron-dense granules; (2) the relatively scarce *b* cells, which are irregular in shape and which contain electron-dense cytoplasmic granules. Neurosecretory axons originating from within the brain pass through the fibrous brain sheath and are found amongst the cells of this epithelium. The endings of other neurosecretory axons are found adjacent to the inner surface of the fibrous sheath that encapsulates the brain and separates it from the epithelium.

Since previous work on nereid reproductive endocrinology had been carried out on oviparous species, it was of special interest to determine if endocrine control of gamete maturation in *N. limnicola,* a viviparous species, conforms to the general nereid pattern. Golding, Baskin and Bern (1968) observed that the infracerebral gland epithelium of *N. limnicola* was thicker than that of other nereids, and speculated that the unusually well-developed infracerebral gland of *N. limnicola* might be related to the viviparous reproductive pattern of this species.

The present paper reports on several experiments related to the endocrine control of reproductive maturation in *N. limnicola.* Furthermore, possible endocrine influence on development of larvae within the coelom was studied. Finally, parts of a severed brain were implanted into decerebrate worms in order to examine the possible relationship of the infracerebral gland to production of the maturation-inhibiting hormone.

Materials and Methods

Specimens of *N. limnicola* were collected at Lake Merced in San Francisco, California. They were gradually adapted to 25% sea water at 14° C in the laboratory for several days before being examined for gametes. This procedure was followed since worms adapted to lower salinities usually did not survive anesthesia. The choice of 25% sea water was made because this salinity falls within the range in which *N. limnicola* regulates the osmotic concentration of its coelomic fluid at levels comparable to that when the worms are adapted to Lake Merced water, which has a salinity of 0.5% sea water and is considered to be fresh (Oglesby, 1965). Each worm was removed from the water and blotted on filter paper. All coelomic fluid samples were of approximately identical volume, and were taken using a fine-tipped capillary tube and examined under a coverslip in a drop of mineral oil. All measurements were taken at 100 × magnification, and oöcyte diameters were measured with an ocular micrometer. As several size classes of

oöcytes were usually present in an individual, only the diameters of the largest oöcytes found in the sample were utilized, following the procedure used by Schroeder (1968). Terminology for the developmental stages and larvae follows Smith (1950).

The quantity of motile sperm present in each worm was evaluated on an ordinal scale of 0 to 5 as follows:

0 no motile sperm present in entire sample.
1 less than 10 motile sperm present in entire sample.
2 less than 10 motile sperm present in an average field.
3 11–20 motile sperm present in an average field.
4 21–50 motile sperm present in an average field.
5 more than 50 motile sperm present in an average field.

Because the sampling procedure usually required several days, the worms were held in a refrigerator (6° C) until all specimens used in an experiment had been examined.

All of the worms used in each experiment, including the intact controls, were anesthetized in 5% ethanol in 25% sea water. Decerebration was carried out as described by Golding (1967a). In the case of decerebrate worms which received an implanted brain, the excised brain was inserted into the coelom by pushing it posteriorly through the wound resulting from the brain removal operation. In experiments in which portions of the brain were implanted, the excised brain was placed on filter paper under a dissecting microscope and the cut made with fine-tipped scissors. The desired portion of the brain was then implanted into the coelom as described above. Single parapodia were removed from each worm to identify individuals. The jaws of worms with intact brains were snipped off at the base to prevent cannibalism. All wounds sealed themselves by contractions of surrounding tissues. After the operations, the worms were placed in 25% sea water which had been filtered, boiled, and cooled, and to which 140 mg/liter streptomycin sulfate (Upjohn) had been added. The worms were maintained in plastic refrigerator dishes containing several layers of glass tubes (as described by Golding, 1967a) at a constant 18° C, and were not fed during the experiments. In all cases, the worms appeared healthy and vigorous up to the completion of the experiments.

The results were analyzed statistically using the non-parametric Fisher exact probability, Chi-square, and Mann-Whitney *U* tests from Siegel (1956).

The brains used for histology were fixed in Helly's fluid, sectioned at 5 μ in Paraplast, and stained with paraldehyde fuchsin (Clark, 1955).

Results

The relationship of somatic and gametic maturation

Several somatic changes that are associated with reproductive maturation of other nereids appear to be delayed in *N. limnicola*. The extensive histolysis of body musculature, which characteristically accompanies nereid gametic maturation (Defretin, 1949), does not coincide with sperm and oöcyte maturation in this species. Our observations indicate that muscular histolysis in *N. limnicola* occurs after the gametes have matured and is correlated with the presence of advanced

larval stages in the coelom. Furthermore, as reported by Smith (1950), the coelomocytes are abundant in the coelomic fluid of worms with mature oöcytes, and the coelomocyte concentration does not decline until after cleavage stages are present in the coelom.

Another aspect of somatic maturation that is associated with the reproductive maturity of nereids is the loss of regenerative ability. Adult worms regenerate a pygidium but only rarely regenerate lost segments (Golding, 1967c). In order to determine if this aspect of maturation is delayed in *N. limnicola,* six specimens containing mature oöcytes (130–160 μ in diameter), but no larvae, were kept for three weeks after removal of about one-half of their posterior segments. Each worm regenerated a pygidium but did not regenerate any segments. These results indicate that regenerative growth does not occur during the later stages of gamete maturation of *N. limnicola.*

TABLE I

The effect of decerebration on gamete maturation

Group	Experiment 1*				Experiment 2**						
	Number of worms		Worms with larvae		Number of worms		Worms with larvae		Worms with motile sperm		Motile sperm average score
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	
Decerebrate	15	14	0	12	18	18	0	16	3	17	3.2
Intact	15	12	0	6	18	17	0	2	2	11	2.1
Implant	15	14	0	1	18	18	0	4	1	11	1.6

* 19 days.
** 3 weeks.

Effect of decerebration on gamete maturation

Experiment 1. Forty-five animals were separated into three groups of equal size. A sample of coelomic fluid was obtained from each animal and both the maximum and mean oöcyte diameter was determined for each worm. The absence of sperm was confirmed for each specimen. The three groups were subjected to the following treatments, respectively: *Intact,* brain left *in situ; Decerebrate,* brain removed; *Implant,* brain removed and implanted into the coelom.

The groups were maintained under identical conditions. After 19 days, further samples of coelomic contents were obtained, and the presence or absence of larvae was noted. The results, summarized in Table I, showed that a significantly greater number of *Decerebrate* worms contained larvae than did the *Implant* group (Fisher, $P < 0.005$). The *Intact* and *Implant* groups also differed in this respect (Fisher, $P < 0.05$), but the difference between the *Decerebrate* and *Intact* groups was not significant.

Experiment 2. In a second experiment, the coelomic contents of 54 worms were examined and three groups of equal size were established as in experiment 1: *Intact, Decerebrate,* and *Implant.* Worms at various stages of maturity were apportioned equally among the three groups. Each group was maintained in a

separate container under identical conditions. After eight days, a small sample of coelomic fluid was taken from each worm and examined for sperm. Three weeks after the initial sample, each worm was re-examined for oöcytes, sperm and larvae.

The results of experiment 2 are shown in Table I. Clearly, significantly more specimens of the *Decerebrate* group contained larvae than either the *Intact* or *Implant* groups (Fisher, $P < 0.001$). There was no difference between the *Implant* and *Intact* groups in this respect. Hence, the precocious appearance of coelomic larvae, which followed brain removal, did not occur if a brain had been implanted into the coelom of the decerebrate worm.

There was no difference among the three groups with respect to the presence of motile sperm after eight days. After three weeks, however, a greater proportion of individuals in the *Decerebrate* group had motile sperm as compared to the *Intact* and *Implant* groups (Fisher, $P < 0.05$). The average scores for quantity of sperm, as shown in Table I, indicate that the *Decerebrate* worms produced more sperm compared to the *Implant* worms (Mann-Whitney, $P < 0.01$). Although the *Decerebrate* group also produced more sperm than did the *Intact* specimens, the scores were not significantly different. Nevertheless, the only strictly comparable groups are the *Decerebrate* and the *Implant*, and the results indicate that brain removal results in the production of abnormally more sperm as well as precocious sperm maturation.

Histology of the implanted brains

At the conclusion of experiment 2, the brain was recovered from the coelom of each worm in the *Implant* group. In each case, the ganglion, which was implanted at the level of the prostomium, was located in the posterior region of the body, and was floating unattached in the coelomic fluid. The brains were intact and showed no pronounced allometric changes, and the eyes were in a relatively normal position. Histological examination revealed that the infracerebral gland epithelium was normal in appearance. A comparison of the implanted and *in situ* brains revealed only subtle differences in the appearance of this epithelium (Fig. 1). There was the impression that, in the case of implanted brains, the *b* cells were more numerous, since they seemed to stain slightly more intensely. However, the differences were not evaluated quantitatively. The *a* cells did not exhibit unusual hypertrophy or atrophy, nor did they show significant storage or depletion of cytoplasmic inclusions, as compared with the *in situ* brains, although the *a* cells of implanted brains sometimes showed a slight peripheral deposition of granular material (Fig. 1A). Further, the implanted brains showed no unusual difference in the stainability of the cells and fiber tracts within the cerebral ganglion.

Effect of decerebration on viviparous development

Three groups of 13 worms each were established as described in the previous experiments: *Decerebrate, Intact* and *Implant*. A parapodium was removed from each worm for purposes of identifying individuals. Initially, all worms had developmental stages present in the coelom; the oldest stages present varied from gastrulae to second-cirrus larvae. However, each group had about the same distribu-

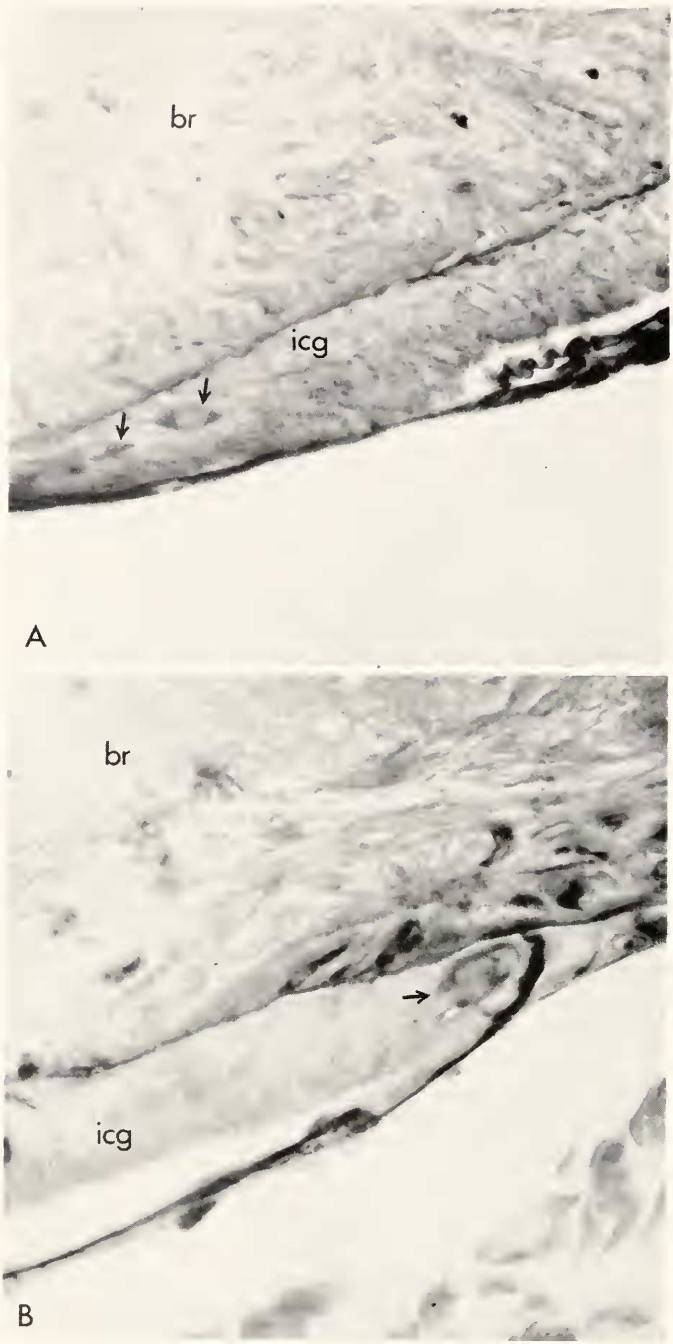

FIGURE 1.

tion of developmental stages. Following the operations, all of the worms were maintained in the same dish containing aerated 25% sea water at 18° C.

After 21 days, the worms were re-examined for larvae; the results are shown in Table II. Coelomic larvae were present in only 45% of the *Intact* group and 66% of the *Implant* group, whereas all survivors of the *Decerebrate* group contained larvae. The *Decerebrate* group thus had a greater proportion of individuals which still had coelomic larvae as compared to the *Intact* (Fisher, $P < 0.01$) and *Implant* (Fisher, $P < 0.05$) groups; there was no significant difference between the latter two groups. However, of those worms which contained larvae in each group, all had larvae and juveniles up to 30 setigers. No worms underwent parturition.

TABLE II

The effect of decerebration on viviparous development

Group	Number of worms		Stages present at conclusion*	Number of worms*	
	Initial	Final*		With larvae	Without larvae
Decerebrate	13	11	19–30 setigers	11	0
Intact	13	11	26–29 setigers	5	6
Implant	13	12	24–30 setigers	8	4

* After three weeks.

Effect of implantation of parts of brains on gamete maturation in decerebrate worms

After initially sampling the coelomic fluid contents of 76 worms, the following groups were established:

Decerebrate Brain removed (15 worms),
Intact Implant Brain excised and implanted into the coelom (16 worms),
Dorsal Implant Dorsal part of the excised brain implanted (15 worms),
Ventral Implant Ventral part of excised brain implanted (15 worms),
Dorsal and Ventral Implant Brain cut and both the dorsal and ventral parts implanted (15 worms).

Initially, all worms contained oöcytes, and the maximum oöcyte diameters varied from 50 μ to 139 μ, but in each group the distribution of worms at various stages of maturity was similar. Sperm were not present in any of these worms. The brain was removed from each worm and, where appropriate, cut in a horizontal plane in order to separate the ventral region, on which is located the infracerebral gland epithelium, from the dorsal part, in which are located many neurosecretory cells. The desired portions were then implanted into the coelom.

FIGURE 1. A. This infracerebral gland epithelium is from a brain recovered after three weeks of implantation into a decerebrate worm. B. This infracerebral gland epithelium is from a normal *in situ* brain; *br*, brain; *icg*, infracerebral gland epithelium *a* cells. The arrows point to *b* cells. (Both A and B: Helly, paraldehyde fuchsin, 500 ×.)

TABLE III

The effect of implanting parts of a brain on gamete maturation in decerebrate worms

Group	Number of worms		Coelomic fluid contents*		
	Initial	Final*	Oöcytes	Larvae	Neither
Decerebrate	15	15	0	11	4
Intact implant	16	16	14	1	1
Dorsal implant	15	12	4	6	2
Ventral implant	15	14	3	9	2
Dorsal and ventral implant	15	15	6	9	0

* After 19 days.

After 19 days, each worm was examined for oöcytes and larvae. The results are summarized in Table III. The *Decerebrate* group had significantly more worms with larvae than the *Intact Implant* group (Chi-square, $P < 0.001$). The *Dorsal, Ventral,* and *Dorsal* and *Ventral Implant* groups were similar in having several worms containing larvae, and thus resembled the *Decerebrate* group in this respect; however, they differed from the *Decerebrate* group in that each of the former had several worms containing oöcytes, but no larvae. The *Intact Implant* group had significantly fewer worms with larvae than did those of the *Dorsal Implant* and *Ventral Implant* groups (Chi-square, $P < 0.01$).

It is conceivable that differences could exist between the groups as to the developmental progress of individual worms, since all stages from cleavage to second-cirrus larvae are included in the category, "larvae." This possibility was examined by considering only the worms containing either oöcytes or larvae (or both). Although larvae of several developmental stages (as well as oöcytes) were often present in an individual, the most advanced stage present was considered an index of developmental progress. The most advanced stages present in individual worms of each group were tabulated and expressed as a percentage indicating the relative proportion of those individuals within each group (Table IV). The data suggest that the decerebrate worms were more advanced with respect to the developmental progress of their coelomic larvae as compared to the other groups, and this impression is strengthened if the groups are compared as to the proportion of each group that reached selected developmental stages, as shown in Figure 2.

TABLE IV

The percentage of worms containing oöcytes or larvae at selected developmental stages for each group in Table III

Group	Oöcytes	Cleavages	Gastrulae	Trochophore	3-segment larvae	Tentacle-bud larvae	2nd-cirrus larvae
Decerebrate	0	0	10	27	18	27	18
Intact implant	93	7	0	0	0	0	0
Dorsal implant	40	0	0	20	10	30	0
Ventral implant	26	8	33	8	17	0	8
Dorsal and ventral implant	40	0	0	13	27	20	0

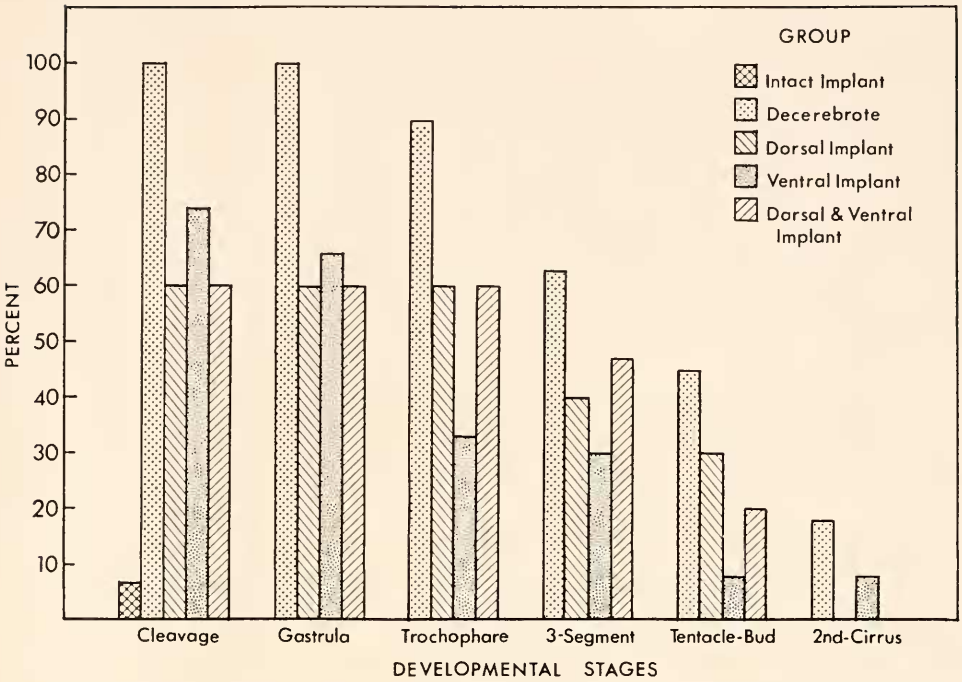

FIGURE 2. This figure compares the developmental progress of the groups in the brain-cutting experiment. The horizontal axis represents larval stages in sequence of development. The vertical axis represents the percentage of worms, in each group, containing larvae which had advanced to the stages indicated. The percentages are based on the worms that contained oöcytes, larvae, or both, for each group, and are adapted from Table IV.

A greater proportion of the *Decerebrate* worms contained larvae which had progressed to each of the selected developmental stages, as compared to the other four groups. Thus, of the worms considered, 90% of the *Decerebrate* group contained larvae which had progressed to the trochophore stage, as compared with 60% for the *Dorsal Implant and Dorsal* and *Ventral Implant* groups and 33% for the *Ventral Implant* group. The one worm which had larvae in the *Intact Implant* group contained cleavage stages; the remaining members of this group contained oöcytes. Although the actual numbers are somewhat small for comparison, the data indicate that the larvae of worms in the *Decerebrate* group showed the most developmental progress, the *Intact Implant* worms showed the least progress, and the three groups which received implants of the brain fragments were intermediate in this respect. Of the latter three groups, the *Ventral Implant* showed the least developmental progress, since a smaller proportion of its members had larvae which had progressed to trochophore or later stages.

DISCUSSION

In many nereids, gametic maturity is accompanied by a profound somatic metamorphosis into an epitokous swimming form known as a *Heteronereis* (Clark,

1961). However, even in species that reproduce in the atokous form, several somatic changes are correlated with reproductive maturation. Dales (1950) has described these changes for *N. diversicolor,* an atokous, oviparous species. The coelomocytes fill the body cavity during the early phase of sexual development and almost disappear during the final stages of gametic maturation as the coelom becomes occluded with mature oöcytes. Histolysis and dedifferentiation of the musculature have rendered the body wall thin and fragile at the time of spawning.

N. limnicola, in common with *N. diversicolor,* reproduces in the atokous form. However, the temporal relationship between events characteristic of somatic and gametic maturation differ significantly in the viviparous *N. limnicola* from the general nereid pattern. Histolysis of body musculature does not coincide with the maturation of the gametes. Furthermore, the coelomocytes fill the coelomic cavity of worms containing mature gametes and the coelomocyte concentration does not decrease until after cleavage stages are present in the coelom (Smith, 1950). The body musculature undergoes histolysis during the period when advanced larval stages are present in the coelom. The coelomocyte concentration decreases significantly by the time of parturition. Thus, the muscle histolysis and the reduction of coelomocytes are delayed to a significant extent with respect to the maturation of the gametes, and occur in correlation with the intracoelomic development of the offspring rather than in association with the final stages of gamete maturation, as is the case with oviparous nereid species.

In *N. limnicola* the gametic and somatic aspects of maturation are out of phase, and this shift can be interpreted in the context of present views of the endocrine integration of nereid reproductive development. Both somatic and gametic maturational changes occur simultaneously in other nereid species, and the declining titer of a single inhibitory hormone is thought to control both processes (reviews by Clark, 1965; Durchon, 1967). The lack of synchrony between these processes in *N. limnicola* raises the possibility that the gametic and somatic aspects of maturation may be controlled by separate inhibitory hormones which are withdrawn simultaneously in an oviparous nereid but withdrawn sequentially in *N. limnicola,* a viviparous species. An alternative and more plausible explanation would attribute this lack of synchrony to different sensitivities of the respective processes to the declining titer of a single inhibitory factor. Thus, somatic maturation may have become more sensitive to the low hormone levels at sexual maturity and thus be delayed with respect to gamete maturation, or the gametes may have developed less sensitivity to the inhibitory hormone and mature precociously with respect to the onset of the somatic changes. It is not possible to decide on one of these alternatives on the basis of present knowledge.

The condition of having the somatic and gametic maturational changes occur out of phase is interpreted as a variation of a basic endocrine control mechanism found in oviparous nereids. In the case of *N. limnicola,* this control is adapted as a specialization for a viviparous mode of reproduction. Thus, the endocrine integration of the process of muscle histolysis, which prepares the sexually mature oviparous nereid for spawning, is modified in *N. limnicola* to prepare the worm for parturition.

With respect to hormonal control of gamete maturation, *N. limnicola* does not appear to depart from the general nereid pattern. The two decerebration experiments demonstrate that brain removal results in the premature appearance of larvae

in the coelom. In experiment 1, the only two strictly comparable groups are the *Decerebrate* and *Implant,* and these give clear indication of an inhibitory influence on gamete maturation and, therefore, indirectly on larval production. The difference between the *Intact* and *Implant* groups is difficult to interpret, although comparable differences have been reported with respect to the progress of regeneration in *N. diversicolor* with intact and implanted brains (Golding, 1967a). This difference is not confirmed by the larger scale experiment 2, but this latter experiment does confirm the results of experiment 1 which demonstrate that the supraesophageal ganglion exerts an inhibitory endocrine control over maturation of the gametes.

Numerous studies have shown that gamete maturation is inhibited by a hormone presumably secreted by the brain, and this inhibition has been described in males (Durchon and Schaller, 1964; Malecha, 1967) and females (Clark and Ruston, 1963; Durchon and Dhainaut, 1964; Durchon and Boilly, 1964; Hauenschild, 1966; Dhainaut and Porchet, 1967) among several species of oviparous nereids. It is not surprising, therefore, that hormonal inhibition of both sperm and oöcyte maturation occurs simultaneously in *N. limnicola,* a self-fertilizing hermaphrodite in which the eggs and sperm develop simultaneously within the coelom of an individual. The maturation-inhibiting hormone is considered identical to the endocrine factor necessary for regeneration of immature worms (Golding, 1967c). Therefore, the observation that sexually-mature *N. limnicola* does not regenerate is further evidence that, relative to immature worms, the titer of the maturation-inhibiting hormone is low in worms with mature gametes.

There has been some doubt regarding the viability of oöcytes whose growth had been accelerated in response to decerebration, since previous workers have failed to obtain normal development following fertilization of these oöcytes (Choquet, 1962; Clark and Ruston, 1963). However, the present results demonstrate that the precociously-mature oöcytes of *N. limnicola,* produced in response to decerebration, are capable of normal development. It is interesting that normal, mature oöcytes of *N. limnicola* resemble the abnormal oöcytes obtained by Clark and Ruston (1963) following decerebration of *N. diversicolor.* In both cases, the oöcytes lack the dense accumulation of yolk droplets characteristic of most nereid oöcytes, which are normally fertilized externally. However, in *N. limnicola* these oöcytes are fertilized in the coelom, and the coelomic milieu undoubtedly provides the nutrients and other factors necessary for the successful growth and development of the embryos. It has been suggested that the infracerebral gland *a* cells may be involved in this respect (Baskin, 1970).

Smith (1950) reported that the oöcytes of *N. limnicola* (from a different population than those used in this study) normally matured at a diameter of 120–170 μ, and our observations indicated that in the worms from Lake Merced, they were fertilized at about 150–160 μ. In the *Decerebrate* group of experiment 2, the oöcytes of worms whose largest oöcytes were between 73 and 128 μ underwent accelerated growth as evidenced by the precocious appearance of larvae in the coelom. It could not be determined, however, whether the oöcytes had attained a normal size at fertilization, or whether they were fertilized at a smaller diameter. One decerebrate worm, whose largest oöcyte were initially about 86 μ, had 148 μ oöcytes after three weeks. Many of the worms which contained early embryonic stages also had oöcytes of 140–160 μ. These observations suggest that the oöcytes accelerated growth to a relatively normal size before being fertilized, rather than

being fertilized at a smaller diameter. It would be of inteerst to know how early the oöcytes of this species can be accelerated by decerebration and still be capable of normal development.

Decerebration of worms with coelomic larvae did not adversely affect the viviparous embryonic development and growth. In fact, the *Decerebrate* group had a greater proportion of worms containing larvae as compared to the *Intact* and *Implant* groups. However, the number of larvae produced by each worm was not recorded, and it is thus conceivable that the groups differed as to the number of larvae produced per worm. The significance of not finding larvae in some of these worms at the conclusion of the experiment is not understood. It is unlikely that the adults underwent parturition, since this event is catastrophic and the adult usually dies. The worms were not fed during the experiment, and it is possible that the larvae were resorbed, since larval resorption has been previously reported in this species (Smith, 1950). Although this question is not resolved, the phenomenon does not alter the general conclusion that larval development *per se* does not require the presence of the cerebral ganglion of the adult.

The results of the experiments in which parts of a brain were implanted into decerebrate worms are significant in view of the problems regarding the presumed source of the cerebral endocrine activity. Previous experimental work has localized this endocrine activity to the posterior region of the cerebral ganglion (Durchon and Dhainaut-Courtois, 1964; Hauenschild, 1966; Golding, 1967b), and attention has been focused upon presumed hormonogenic neurosecretory cells. In contrast, Golding, Baskin and Bern (1968) and Dhainaut-Courtois (1968a) have suggested that the infracerebral gland, which is located on the postero-ventral surface of the ganglion, may fulfill this endocrine role. The present histological observation that the infracerebral gland epithelium was present and normal in appearance on implanted cerebral ganglia that had been secreting the maturation-inhibiting hormone, is compatible with the latter view.

Parts of a brain were implanted into decerebrate hosts to determine whether the source of the maturation-inhibiting hormone is associated with the dorsal region, in which are located many neurosecretory cells, or the ventral region, on which is located the infracerebral gland epithelium. Of the three groups which contained implanted brain fragments, developmental progress was essentially similar in each. As compared to the *Dorsal Implant* and *Dorsal and Ventral Implant* groups, the *Ventral Implant* group showed slightly less developmental progress, which might indicate that the ventral region of the brain exerts a stronger inhibitory effect on maturation. But an equivalent effect was not obtained when a ventral and dorsal part were implanted together; thus, any conclusions about a differential influence of the dorsal and ventral regions, respectively, are considered tentative.

The inhibitory effect of the brain fragments was not equivalent to that of an intact ganglion, even where dorsal and ventral fragments of the same brain were implanted together. But neither were the fragments totally without effect, since all three groups which received an implanted brain fragment showed evidence of developmental retardation when compared to the decerebrate group. This retardation is considered an indirect effect stemming from the inhibition of oöcyte maturation, and does not suggest a direct inhibitory influence on larval development.

Although this experiment did not localize the source of the hormone, the results are consistent with the hypothesis that neurosecretory cells of the brain and the cells of the infracerebral gland epithelium may form a functional unit or system for the production of the maturation-inhibiting principle. The physical integrity of this system was apparently disrupted by transecting the brain so as to separate the infracerebral gland epithelium from its neurosecretory component, resulting in the reduction, but perhaps not complete absence, of hormone secretion.

This interpretation does not conflict with the view that the infracerebral gland cells may be endocrine, although their significance in this respect is incompletely understood. While the *a* cells of the infracerebral gland epithelium have been compared in their ultrastructure to that of endocrine cells of the insect corpus allatum (Dhainaut-Courtois, 1968a), it has been concluded elsewhere that the *a* cells are probably not the source of known endocrine principles (Baskin, 1970). However, the granule-filled *b* cells, which resemble protein-secreting gland cells in their ultrastructure, are most differentiated in immature worms and degenerate in older worms (Dhainaut-Courtois, 1968a). Since this degeneration parallels the disappearance of the maturation-inhibiting hormone, it is tempting to speculate that the *b* cells may be associated with the secretion of this hormone.

The functional significance of the infracerebral gland epithelium and its relationship to neurosecretory activity of the brain remain to be elucidated. Nevertheless, future investigations on nereid endocrinology must consider the possible importance of this system to neuroendocrine regulation in these worms.

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Summary

1. The relationship of gametic and somatic maturation in the atokous polychaete, *Nereis limnicola,* differs significantly from the general nereid pattern. In this viviparous species muscle histolysis and the decrease in the coelomocyte concentration are delayed with respect to the onset of gamete maturation, and occur, instead, in association with the intracoelomic development of the offspring. However, worms with mature gametes are unable to regenerate lost caudal segments.

2. The cerebral ganglion of *N. limnicola* exerts an inhibitory influence on the maturation of sperm and oöcytes. Brain removal results in the precocious appearance of normal larvae in the coelom. This effect is prevented by implanting a brain into the coelom of the decerebrate worms.

3. Histological examination of brains that had been implanted for three weeks revealed that the infracerebral gland epithelium was present and normal in appearance.

4. Decerebration of worms containing coelomic larvae did not delay or accelerate larval development.

5. The effect of implanting parts of brains on gamete maturation of decerebrate worms was studied, and the results indicated that the dorsal part of the brain, which contains many neurosecretory cells, and the ventral part of the brain, on

which is located the infracerebral gland epithelium, had approximately equivalent inhibitory activity when implanted alone or together, but this inhibitory effect on oögenesis was not as great as that produced by an implanted intact brain. These results are interpreted to mean that the infracerebral gland epithelium and neurosecretory cells of the brain may form a neuroendocrine system, the integrity of which is essential for secretion of the maturation-inhibiting hormone at normal levels.

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